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## The ongoing transition at an exponential speed from Conservation genetics to Conservation genomics

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### Abstract

Conservation genetic disciplines have greatly progressed during the last thirty years, mainly thanks to the continuous development of molecular biological knowledge and the implementation of molecular tools used to describe diversity at the DNA level. The ongoing transition from Conservation genetics to Conservation genomics is showing to increase at an exponential speed as the integrated use of various kinds of molecular genetic data and bioinformatic approaches may improve our theoretical knowledge and practical approaches in the conservation and wise use of biodiversity. Aim of this mini-review is to push forward the ongoing transition, bearing in mind that most of the applied conservation programs would not need entire genomic data set, which are still expensive and time consuming.

**Keywords:** hybridization, introgression, inbreeding, outbreeding depression

### Introduction

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### Introduction

#### *State of the art*

The effects of environmental changes (eg. climate induced changes) in natural ecosystems are diverse, often complex, and unpredictable and have several consequences at various scale for biodiversity, for example; changes of the degree of species interactions, changes of the phenology, and changes of

species distributions and abundances (Walther et al., 2002; Pertoldi et al., 2007a,b; Ouborg et al., 2010). All these consequences influence indirectly the genetic variability of the populations (Pertoldi et al., 2007a). Michael E. Soulé and colleagues in their seminal collaborative books (Soulé & Wilcox, 1980; Soulé, 1986) defined Conservation Biology as a “*science of crisis*” that originated from the awareness of the dramatic loss of biodiversity that has occurred since the second half of the last century. The destruction of natural habitats, profound landscape transformations, overexploitations of forests, hunting and persecution of animal populations was continuing for centuries in most of the European countries and around the Mediterranean basin where the loss of biodiversity was highest following the first industrial revolution. More recently, post-colonization economic developments in Asian and South American countries rapidly worsened the conservation *status* of natural areas particularly in consequences of deforestation and the spread of agricultural lands. Last but not least, the globalization of economies, trades and travels dramatically increased the worldwide diffusion of alien invasive species (Luque, 2013). Conservation biologists realized that science should rapidly develop novel technical and communication tools to contribute to halt the loss of biodiversity, and that those contributions should be inherently interdisciplinary (Soulé & Wilcox, 1980). Indeed the two books: *Conservation Biology. An evolutionary-ecological perspective* (Soulé & Wilcox, 1980), and *Conservation Biology. The science of scarcity and diversity* (Soulé, 1986), included contributions from plant and animal ecologists, demographers, pathologists, evolutionary biologists and population geneticists.

In fact, for the first time after Frankel’s paper (*Genetic Conservation: our evolutionary responsibility*; 1974) and Frankel & Soulé’s book (*Conservation and evolution*; 1981) the genetics of populations and the principles of evolutionary biology were firmly included within the sciences of biological conservation, thus leading to a definition of Conservation Genetics as: “*the theory and practice of genetics in the preservation of species as dynamic entities capable of evolving to cope with environmental change to minimize their risk of extinction*” (Frankham, Briscoe & Ballou, 2002).

Conservation genetic disciplines have greatly progressed during the last thirty years, mainly thanks to the continuous development of molecular biological knowledge and the implementation of molecular tools used to describe diversity at the DNA level. Progress in laboratory equipment and information technology has made it possible to apply increasingly sophisticated and powerful molecular and computational procedures. First-generation automated sequencers, Bayesian statistical methods and simulation algorithms boosted the production and accurate analyses of large empirical data sets. Conservation genetics aims at contributing to solve some fundamental issues. First of all, the reliable assessment and conservation strategies of genetic diversity (allelic diversity and heterozygosity) in small fragmented populations and endangered species (Frankham, 2010). Then, the assessment of inbreeding and its consequences, inbreeding depression, in natural and captive populations. Translocation and artificial gene flow strategies have been implemented aiming at reducing the deleterious consequences of inbreeding in small isolated natural populations (rescue effects; Tallmon et al., 2004). The management of pedigrees and purging strategies have been implemented to purge, as far as possible, the genetic load in inbred captive populations (Leberg & Firmin, 2007). In contrast, the theory, prediction and assessment of the risks of outbreeding depression, a likely consequence of crossbreeding or artificial gene flow among genetically divergent parentals or populations, are still definitely unexplored (Frankham et al., 2011). Understanding the processes of adaptation to captivity and selection for domestication are particularly important to fishery and hatchery culture (Frankham, 2008). It is difficult to estimate the extent of quantitative genetic variation and its dynamics in natural populations of endangered species. Thus a main issue in conservation genetics is to understand the reliability of estimates of molecular variation (heterozygosity) as proxy to quantitative traits variation and eventually to fitness (Reed & Frankham, 2003; Pertoldi et al., 2007a,b).

This issue is crucial to implementing procedures for a reliable assessment of the potential for adaptation and evolution, which should rely on accurate discovery of the genotype-phenotype-fitness connections. The identification of evolutionary lineages and the resolution of taxonomic uncertainties has been a main contribution of molecular systematic and taxonomy to conservation biology. However, this issue

has always been confronted with the problematic multiple definitions of species and their difficult uses in practical conservation biology (Frankham et al., 2012).

Another major contribution of population genetics to the conservation of biodiversity has been the definition of intra-specific evolutionary significant units (ESUs) and management units (MUs) (Funk et al., 2012). The assessment of rapid micro-evolutionary changes and particularly those generated by anthropogenic processes, such as the consequences of global climate changes, the overexploitation of marine and terrestrial species, the ecological competition and hybridization due to the diffusion of feral domesticated animals and alien invasive species, is now becoming a priority concern in conservation biology (Randi, 2008). All these issues have boosted both theoretical and practical applications in many fields of natural and captive population management (Frankham, 2010).

The success of the Conservation genetics vision is largely based on the widespread and successful use of molecular markers and standard population genetic models. Both are powerful, but not without their own weaknesses. Basic assumptions in conservation genetics are that molecular markers are selectively neutral, they are in Hardy-Weinberg and linkage equilibrium (HWLE). The most frequently used molecular markers are limited numbers of hypervariable autosomal microsatellite loci (Short Tandem Repeats – STR). Most of the published studies used from c. 10 to a few dozen of STRs, often selected opportunistically, that is based on the public availability of their PCR primer sequences and amplification protocols, their polymorphisms in the target populations, and low costs of the laboratory analyses. STR selective neutrality has always been assumed, but very rarely tested. Lack of STR functionality has also been assumed, based on the standard “dogma” of molecular biology (one coding DNA gene - one transcript RNA - one functional protein molecule), but it has never been verified. In fact, recent studies have shown functional roles of STR expansion-contractions in regulating gene activities, and, in general, have revealed that most of the so-called “junk” DNA may have essential regulatory functions (Haas & Payseur, 2012). Formal tests of HWLE on STR panels rely on unrealistic standard population genetic models (e.g., the sampled populations should have “infinite” size) or have low power to detect departures from the equilibria (e.g., Bonferroni or similar adjustments for replicated assays). Short sequences of mitochondrial DNA genes (mtDNA), often the hypervariable domains of the control-region (CR), the protein-coding cytochrome *b* gene (CYTb) or the barcoding marker cytochrome oxidase I (COI), are used in population genetic and phylogeographic studies. These maternal sequences are used assuming neutrality, which is often unknown or not true (Betancourt et al., 2012). Moreover, sequencing the entire mtDNA genomes often produces results that are discordant with those resulting from the analyses of short sequences and the use of paternally inherited chromosome Y-linked DNA sequences (or Y-linked STR) is still very limited in conservation genetics (Schregel et al., 2015). The use of autosomal functional gene sequences (e.g., the MHC genes) is also limited, and results are often unclear or inconclusive (Galaverni et al., 2015). The development of coalescent modelling and Bayesian statistical methods allowed investigators to avoid some of the unrealistic equilibrium assumptions of standard population genetics, but they nevertheless require a larger data set than that usually obtained through molecular marker procedures (Aeschbacher et al., 2012).

#### *From Conservation genetics to Conservation genomics*

Conservation genetics has been successful in highlighting the roles of evolutionary and population genetics for the conservation of biodiversity and the sustainable use of biological resources, but it has not fully been able to resolve crucial issues such as:

- 1) How many molecular markers are needed for a reliable representation of the heterozygosity of each individual, the genetic variability in a population, the genetic distances among populations, and the patterns of interspecific phylogenetic divergence?
- 2) How can we use estimates of heterozygosity based on small numbers of molecular markers as a proxy of fitness?
- 3) What are the relations among molecular marker variation, phenotypic variability and evolutionary potential?

- 4) Are we only assuming, or could we efficiently test, if limited panels of molecular markers are selectively neutral (thus informative to reconstruct the population and demographic history of the studied populations), or not (thus informative to decipher past or recent selective processes)?
- 5) When multiple-gene phylogenetic trees are reliable enough to become species trees?
- 6) Are limited numbers of markers able to identify past generation hybrids and describe complex processes of introgression?
- 7) Can we predict inbreeding/outbreeding and its consequences – inbreeding/outbreeding depression – from limited numbers of molecular markers?

These and other crucial questions could be better explored and answered by genomic approaches applied to conservation (Pertoldi et al. 2007a; Allendorf et al. 2010; Ouborg et al. 2010). Conservation genomics has become possible by the rapid development of DNA sequencing technology, which moved from the Sanger-based chemistry and electrophoretic separation of DNA fragments, to novel non-Sanger sequencing and detection methods mainly derived from real-time PCR protocols. The next-generation sequencing methods (NGS) allow relatively rapid and massive generation of DNA sequences that, coupled with intensive bioinformatic data analyses, could, in principle, allow reconstruction of entire genomes in reasonably short time and with limited costs (Angeloni et al., 2011). However, genomics does not mean simply more data. Genomics means tremendous opportunities to reconstruct the architectures of individual genomes and patterns of multiple locus interactions in the evolutionary dynamics of individuals, populations and species. Genomic platforms can be used in their full or reduced potentialities to generate exhaustive descriptions of genetic variability and genomic architecture in both model and non-model species. In this way genomics can help answer some of the crucial issues conservation genetics was able to highlight but not to resolve.

#### *Identification of selected vs. neutral DNA sequences or chromosomal regions*

Comparative analyses of entire genomes or widespread chromosomal markers stimulated the development of computational approaches to identify genomic regions that have been shaped by various kinds of natural selection pressures (Nielsen, 2015). Selected markers are identified as outliers in the background of neutral genomic variation (Foll & Gaggiotti, 2008). Outlier loci can then be associated with specific environmental variables (Joost et al., 2007). Gene network and ontology analyses led to identification of enriched clusters of candidate genes showing similar or epistatic functions (Khatri & Draghici, 2005). Recent highly efficient gene editing methods could be designed to knock-out or modify candidate genes with known phenotypic effects, thus allowing experimental analyses of the gene – phenotype functional connections also in non-model species (Bono et al., 2015). In this way it is possible to identify those DNA sequences that effectively behave neutrally, and that can be used in population genetic analyses to describe the genetic consequences of demographic processes (effective population size and random genetic drift; dispersal and gene flow across population clusters and metapopulations). In contrast, functional genes which underwent selective processes can be used to evaluate the historical or contemporary consequences of adaptation.

#### *Reliable estimates of historical and current effective population size and gene flow*

The identification of millions of neutral markers in entirely sequenced genomes makes it possible to obtain accurate estimates of effective population size ( $N_e$ ) and migration rates ( $m$ ). These parameters can be correctly estimated using exclusively the neutral subset of genome markers and avoiding the confounding effects of non-neutral outlier DNA sequences. In fact, coalescent theory and models clearly show that selected loci can compress the gene genealogies (in case of selective sweep or hard directional selection) or can expand the genealogies (in case of balancing and disruptive selection). Historical dynamics of population expansion or decline can be efficiently estimated analyzing the patterns of heterozygosity even in a single genome (Gronau et al., 2011). The size and timing of population bottlenecks can be accurately estimated. Moreover, genomic data sets allow detailed analyses of linkage,

which have been virtually impossible with limited molecular marker panels. Analyses of haplotypes have been used to infer details on asymmetric or sex-biased patterns of gene flow (Schregel, 2015).

#### *Inference of population structuring*

Powerful Bayesian models have contributed to reconstruct the patterns of cryptic population structuring, i.e., that is to identify any number of a-priori unknown genetically distinct populations (Pritchard et al., 2000). Population clusters are then used to assign individuals of unknown origins, migrants or hybrids to their parental populations. In this way it is possible to identify cryptic populations that can be eventually classified as novel species, subspecies or ESUs, and estimate accurately ongoing rates of gene flow and admixture (Leachè et al., 2014). The Bayesian clustering models and novel multivariate procedures (Jombart, 2008) strongly improved the identification of genetic units, but they need wide empirical set of markers, which can be obtained through genomic analyses. Also in these case-studies, the accurate distinction of neutral vs. non-neutral markers is crucial. For instance, populations of common species did not diverge by drift because of their large effective population size. Absence of genetic differentiation at neutral markers, however, does not mean that local populations are connected and completely admixed, but simply that those populations could not diverge by drift. In contrast, selected loci can display significant signature of local adaptation, and can be used to identify distinct stocks that are demographically independent (Milano et al., 2014).

#### *Admixture analyses and introgression*

The consequences of hybridization and crossbreeding between individuals belonging to genetically differentiated populations can be fully identified only through the use of many molecular markers (Randi et al., 2001, 2002). It is well known that after the first two-three generation of hybridization and introgression hundreds of unlinked neutral markers are needed to identify the genomic classes of the admixed individuals. Genomic data can be used to identify the parental population of origin of admixed genotypes. Moreover, genotypes can be phased and the reconstructed haplotypes can be used in linkage analyses to improve genome wide introgression analyses (Lawson & Falush, 2012). Detailed reconstructions of the patterns of linkage decay after initial hybridization, identification of haplotype blocks and runs-of-homozygosity can supply detailed information on introgressed chromosomal segments that are eventually positively selected (or, in contrast, selectively purged). Reconstructed recombination patterns have also been used to estimate the time since admixture in hybridizing populations (Lawson & Falush, 2012; Iacolina et al., 2016).

#### *Inbreeding depression*

Standard population genetic models and empirical sets of data based on limited number of molecular markers completely failed to identify genes related to inbreeding and predict inbreeding depression. Genomic data allow fine-scale mapping of functional genes and variants in inbred familial groups. In this way inbreeding genes, deleterious allelic variants and mechanisms controlling inbreeding depression can eventually be identified (Kristensen and Sørensen, 2005; Kristensen et al., 2010; Reed et al., 2012).

#### *Applied conservation genomics*

“*Conservation genomics*” can be broadly defined as the use of new genomic techniques to solve problems in conservation biology (Allendorf et al., 2010). Genomic platforms and bioinformatic tools are rapidly improving, allowing fast accumulation of huge datasets at increasingly lower costs also in non-model plant and animal species. Obviously, conservation sciences will benefit enormously from the use of genomic technologies and resources.

#### *Whole genome sequences*

Publicly available entire genomes of reference model or non-model species are essential to describe the architecture of species' genetic variation and select the most informative markers. The genomes should have been accurately reconstructed and genes should be accurately identified, mapped and annotated.

Comparative whole-genome sequencing is the essential starting step to identify neutral DNA sequences as well as candidate loci. Whole genome data integrated with transcriptome, proteome and metabolome data will predictably provide in the near future the avenues to decipher the gene-to-phenotype functional connections. A number of molecular techniques (GBS, RAD-tag, ddRAD sequencing) yield reduced subset of target sequences which simplify the generation of data at lower costs (Narum et al., 2013, Bahrndorff et al., 2016). Reduced representation methods can generate thousands to hundreds of thousand SNPs at homologous DNA sequences in hundreds of samples, thus enabling immediate population genetic analyses and the identification of informative markers. The DNA reads produced by reduced representation methods can be easily aligned and mapped into the available reference genomes, thus allowing the identification of synonymous vs. non-synonymous polymorphisms in protein-coding genes, or SNPs in regulatory regions. Linkage analyses allow the identification of haplotype blocks and ROH, that are extremely informative in admixture analyses. The bioinformatic evaluation of whole genome or reduced representation sets of data can result in the accurate selection of informative panels of molecular markers, usually SNPs, but also STRs or indels. Panels of selected informative markers can be used to genotype population samples at low cost in conservation genetics or monitoring programmes. Tens to hundreds of thousands of SNPs are spread in DNA microarrays, currently available in a growing number of plant and animal species. Custom SNP panels can be easily designed and analysed in low-cost microfluidic platforms (Wang et al., 2009; Mikheyev & Tin, 2014). The fast technical progresses in genomic engineering are producing equipments that promise efficient and cheap applications also in practical conservation genetics. Personal genomic platforms can be used to sequence small genomes, multilocus amplicons, DNA sequences from capture arrays and other DNAs obtained through reduced representation methods (Wang et al., 2009; Mikheyev & Tin, 2014). Other useful platforms in conservation are real-time PCR procedure implemented in small volumes of 386 microwell plates, and microfluidic chips (Wang et al., 2009). These platforms are flexible enough to analyze at low cost different arrays of sample x custom marker numbers, thus providing the data normally used in conservation and monitoring programmes. In parallel, bioinformatic software to analyse genomic data are becoming increasingly user-friendly.

## Conclusions

Undoubtedly, we are assisting to a transition from the conservation genetics to the conservation genomics era. Further scientific progress will be accelerated by merging and complementing current efforts in evolutionary and ecological genetics by: 1) collecting informative genetic and environmental data sets in natural populations and from preserved specimens, 2) merging taxonomic, ecological and genetic databases 3) using molecular data in synergy with quantitative traits and environmental data, 4) unravelling the distribution of variation at functional vs. non-coding sequences in natural populations.

However, the evaluation, validation, and implementation of new molecular and theoretical tools have to be further developed in order to standardize research approaches and evaluation procedures,

Lastly, we should always bear in mind that most of the applied conservation programs would not need entire genomic data set, which are still expensive and time-consuming. Most of the ongoing conservation programs would, however, strongly benefit from accurate selection of large sets of informative markers. These panels can be obtained through adaptive handling of the above mentioned pipelines.

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